# Prospects for a Prophylactic HPV Vaccine:
## Rationale and Future Implications for Cervical Cancer Screening

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*Cytologic screening in combination with ablative therapy has helped reduce cervical cancer mortality in the developed world. Despite the success of this approach, cervical cancer remains a major cause of death, especially among women with limited access to health care. Recognition that human papillomaviruses (HPVs) are the main etiologic agent in cervical cancer suggests that a prophylactic vaccine could reduce the incidence of HPV infection and, therefore, achieve cancer control with reduced reliance on costly screening programs. In this review, the rationale for developing a prophylactic HPV vaccine and the potential impact that vaccination would have on cervical cancer screening are discussed.* Diagn. Cytopathol. 1998;18:5–9. 

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Cervical cancer prevention programs based on cytologic detection of cancer precursors, with ablation of biopsy-confirmed lesions, have helped reduce cancer mortality throughout the industrialized world. However, the cost-effectiveness of this approach and the morbidity associated with overzealous treatment of minor precursor lesions have raised increasing concerns. Use of new screening techniques may improve the management of cervical disease, but the impact of these innovations on cervical cancer mortality will be limited because most women who die of cervical cancer have not been regularly screened. To achieve a significant improvement in cancer control worldwide, a strategy which focuses on reducing cancer risk, rather than one which relies on multiple screening and treatment interventions, is needed.

Recognition that human papillomaviruses (HPV) represent the etiologic agent in nearly all cervical carcinomas and squamous intraepithelial lesions[1,2] suggests that cervical cancer rates could be sharply reduced by preventing HPV infection through prophylactic immunization. An effective vaccine could reduce the need for expensive screening programs and would be especially beneficial in countries with the highest cancer mortality rates and the most limited economic resources. A number of different vaccine strategies are currently under consideration. The current discussion will focus on the prospects for developing a prophylactic HPV vaccine, with special emphasis on the potential impact that a successful vaccination program would have on cervical cancer screening. Readers may consult recent reviews summarizing efforts to develop a therapeutic HPV vaccine.[3–5]

Four observations underlie the rationale for developing a prophylactic HPV vaccine: 1) cervical cancer is linked to sexually acquired HPV infection; 2) HPV is etiologically implicated in nearly all cervical cancers; 3) relatively few HPV types account for the majority of cervical cancers; and 4) host immune responses to HPV appear to be important in preventing the progression of HPV infection to clinical disease.

### Evidence That Cervical Cancer Is Related to Sexual Transmission of HPV

The epidemiology of cervical cancer and HPV infection has been recently reviewed.[6] The observation that cervical cancer is common in prostitutes, but infrequent in nuns and monogamous women, is one of the earliest findings linking cervical cancer to a sexually transmissible agent. Recognition that cervical cancer rates are increased among women whose spouses have penile cancer provides additional support for the role of a transmissible agent in cervical carcinogenesis. In addition, formal epidemiologic studies have consistently demonstrated that contact with multiple sexual partners and early age at first intercourse represent the strongest risk factors for cervical cancer and its precur-

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sors. More importantly, the increased cervical cancer risk that results from sexual behavior is mainly a reflection of the likelihood of acquiring HPV infection.[2] Recently, investigators also showed that monogamous women whose partners frequent prostitutes have rates of cervical cancer comparable to those of women with multiple partners, again linking cervical cancer to a sexually transmitted agent, specifically HPV.[7] Finally, serologic studies using reliable methods have demonstrated that antibodies recognizing HPV antigens are generally absent in virgins and appear with sexual debut.[8] In summary, a link between cervical cancer and a sexually transmitted agent has been recognized for decades; the availability of reliable molecular techniques has convincingly demonstrated that the agent in question is HPV.

## Evidence That HPV Is the Etiologic Agent in Cervical Cancer

Multiple studies demonstrate that detection of HPV DNA is strongly associated with cervical cancer and squamous intraepithelial lesions (SIL). HPV infection precedes the development of SIL and persists at the time of diagnosis. HPV DNA, mRNA, and proteins have been demonstrated in the vast majority of SIL and carcinomas tested. Furthermore, in vitro studies demonstrate that HPV, in combination with other factors, is capable of immortalizing or malignantly transforming cells in culture. Finally, the E6 and E7 proteins of cancer-associated HPV types interfere with the function of the tumor suppressor genes p53 and retinoblastoma, providing a plausible mechanism linking HPV infection to cervical carcinogenesis.[9,10]

## Specific HPV Types Found in Cervical Carcinoma

Approximately 25 of the more than 70 HPV types that have been described are identified in the cervix. HPV types are classified as ''low-risk'' or ''cancer-associated'' based on the diseases in which the specific viruses are most commonly found. Low-risk viruses are the primary cause of condyloma acuminatum and are also found in some low-grade SILs, but are rarely identified in women with high-grade SIL or carcinoma. Cancer-associated types represent the primary etiologic agent in cervical carcinoma and high-grade SIL and also account for the majority of low-grade SIL. HPV DNA has been detected in approximately 93% of 1,000 invasive cervical carcinomas collected in 22 countries throughout the world.[1] More than 20 different HPV types were identified in these specimens, but HPV types 16, 18, 31, and 45 were found in over 75% of the tumors. The type distribution varied little between countries, with HPV 16 accounting for about 50% of the carcinomas. Thus, it appears that many viruses are associated with cervical cancer, but a relatively small number account for the vast majority of tumors.

## Immune Responses in Women With HPV-Associated Lesions

Clinical observations provide compelling evidence for the importance of the immune system in determining the outcome of HPV infections. For several years, it has been recognized that recurrent genital warts and increased rates of SIL and carcinoma are observed among women diagnosed with several conditions associated with immunodeficiency, including transplantation, lymphoma, and human immunodeficiency virus infection.[11] Histopathological observations support the role of the immune system in controlling HPV infection. Dense infiltrates of T lymphocytes and macrophages have been described in regressing warts, whereas Langerhan's cells (antigen-presenting macrophages) are reduced in SIL. These observations have led to more intensive investigations of immune responses to HPV.

Papillomaviruses (PV) elicit both humoral (antibody-mediated) and cell-mediated immune responses. These reactions are restricted by major histocompatibility complex expression (HLA class I and II antigens) and reflect the function of many different cytokines and immune effector cells. Several interesting observations have been made concerning HLA expression, including the identification of a possible association between specific HLA types and increased rates of carcinoma.[12] In addition, it has been noted that HLA class I molecules are frequently downregulated in cervical carcinomas, and that expression of class II molecules may be increased in SIL and carcinoma compared with normal tissues.[11] These findings suggest that the interaction between HPV and host target cells may be an important determinant of the effectiveness of the host immune reaction. Although cellular immune responses may be related to the effectiveness of a prophylactic HPV vaccine, the importance of cellular immunity has received more attention in the context of therapeutic HPV vaccines.

The kinetics and duration of serologic responses to HPV antigens are complex and have not been completely delineated.[8] Different immunologic markers correlate with the presence or absence of SIL, carcinoma, or HPV DNA detection. The failure to detect HPV antibodies in women with detectable HPV DNA may reflect the time at which the sample was obtained (before the antibody appears or after titers have declined to undetectable levels), general anergy, specific immune tolerance, the choice of serologic test, or other factors. The detection of antibodies to HPV proteins in women with cancer or SIL indicates that not all antibody responses are protective. However, specific neutralizing antibody responses to HPV structural capsid proteins (L1 and L2) appear to protect against papilloma formation following experimental viral challenges in animal models, suggesting that the development of a prophylactic HPV vaccine is feasible.

## Challenges in the Development of Papillomavirus Vaccines

HPV vaccine development has been impeded by the lack of an abundant source of HPV antigens. Human cervical lesions contain few virions, and cancer-associated PV cannot be grown using standard culture techniques. Because PV infections are species restricted, it is impossible to study HPV infection in laboratory animals. Therefore, many vaccine studies have been performed using cottontail rabbit PVs,[13–17] bovine PVs,[18–20] and canine oral PVs[21,22] in animal models. These PV, like HPV, produce warts with some potential to develop into cancer. However, the natural history of HPV and animal PV is not identical. Therefore, extrapolating from animals models to clinical medicine requires caution.

Most prophylactic vaccine studies have used the L1 and/or L2 proteins as antigenic targets. L1 composes about 80% of the viral capsid (protein coat), with L2 comprising the remainder. PV vaccines against L1 and L2 have been prepared using fusion proteins, vaccinia virus recombinants, plasmids, virus-like particles (VLPs), and other methods. VLPs are spherical 50-nm structures resembling hollow viral capsids that are produced using molecular techniques.[23] VLPs possess structurally intact viral capsid proteins which elicit protective antibody responses in animals. Because VLPs lack oncogenic DNA and can be produced in abundant quantity, vaccination with VLPs is attractive. In addition, VLPs may be used in enzyme-linked immunosorbent and hemagglutination inhibition assays to detect humoral responses to HPV.

## Animal Models

Studies performed in animals reveal several consistent findings relevant to the development of a prophylactic HPV vaccine.[13–22] Vaccination with PV capsid antigens evokes a neutralizing antibody response which protects animals against experimental viral challenge. In rabbits, immunization against L1 also prevents latent infection with cottontail rabbit PV.[14] Compared to control animals not receiving PV vaccines, the papillomas which form in vaccinated animals are generally smaller, display a greater tendency to regress spontaneously, and do not progress to cancer. Vaccination with L1 alone is protective in most systems. Protection against experimental viral challenge is species- and type-specific and requires the use of vaccines containing structurally intact proteins. To date, vaccines prepared from linear epitopes, protein fragments, and denatured proteins have not been protective. Finally, administration of vaccines via an intramuscular or subcutaneous route appears to provide mucosal protection. Recent results obtained with vaccines against canine oral papillomavirus (COPV) are particularly significant and are presented in greater detail.

## Canine Oral Papillomaviruses

Bell et al.[21] demonstrated that vaccines prepared from formalin-fixed COPV wart extracts protected beagles against oral challenge with COPV. In this study, 99 dogs that received intradermal footpad injections of COPV vaccine at age 8 and 10 wk were given oral COPV challenges 1 mo after vaccination and were then observed for 12 wk. Oral papillomas developed in all 26 control animals injected with saline at 6–8 wk, whereas none of the vaccinated dogs developed oral lesions. Following this experiment, a routine vaccination program was initiated. Immunization of approximately 60,000 beagles over a 3-yr period resulted in complete protection against naturally acquired COPV-induced warts. These data suggest that a formalin-inactivated vaccine is highly protective against both natural and experimental COPV infection.

Suzich et al.[22] demonstrated that a vaccine prepared from COPV L1 VLP protects dogs against experimental COPV challenge and evokes a neutralizing IgG serum response. Vaccination with denatured COPV L1 VLP was not protective, demonstrating that presentation of structurally intact proteins to the immune system is required to elicit a protective antibody response. Vaccination with HPV 11 L1 protein was not protective, demonstrating that the immune responses to PV proteins are species-specific. Because naive dogs transfused with serum IgG obtained from vaccinated dogs were protected, it appears that humoral responses are sufficient to prevent PV infection, despite the probable importance of cell-mediated immunity in viral clearance. In addition, these investigations demonstrate that vaccination with L1 alone may be protective and that the addition of L2 or other PV proteins is not essential for successful prophylactic vaccination. The use of adjuvants increased the duration of antibody responses, but did not alter peak antibody levels.

## Considerations for the Development of a Prophylactic HPV Vaccine

It is likely that early vaccination programs will target HPV 16, 18, 31, and 45 because these agents cause the majority of human carcinomas. It is unknown whether vaccination against a specific HPV type would confer protection against related viruses, but based on results in animal models, it is likely that vaccination against HPV capsid proteins would provide type-specific protection. Using recombinant DNA technology, it should be possible to prepare a safe HPV vaccine, free of contamination with potentially oncogenic DNA and protein. Questions concerning dosage, adjuvants, boosters, and other issues related to administration are still under consideration. Experimental data suggest that systemic immunization may be protective.

Selecting the appropriate target population represents another critical issue. Because most HPV infections appear to be acquired through sexual contact, vaccination of adolescents prior to the initiation of sexual activity would

appear to offer the best opportunity to prevent HPV infection. Based on studies suggesting that men with many sexual partners confer risk on their partners,[7] it may be useful to vaccinate members of both sexes, even though clinically significant HPV-related diseases afflict mainly women. The utility in vaccinating women who are currently HPV DNA-positive or who have been treated previously for HPV-related diseases is unclear.

## Implications for Cervical Cancer Screening

Implementation of a prophylactic HPV vaccination program would have important implications for cervical cancer screening. During the initial period following the introduction of a vaccine program, the population will include both vaccinated women at low risk for cervical neoplasia and women who have not been vaccinated who will be at greater risk. If initial efforts focus on vaccinating at-risk adolescents, risk profiles may vary in women of different ages.

## Cervical Cancer Prevention in Younger Women

Most likely, young women would be vaccinated before initiation of sexual activity. The development of an appropriate serologic test to document that vaccination resulted in a protective immune response will be critical. The approach to screening this low-risk group following vaccination is unclear. Several factors would need to be considered.

It is unlikely that a vaccine directed against the most common HPV types could entirely eliminate cervical cancer in a population because many HPV types are found at least occasionally in these tumors. Therefore, cancer risk could be reduced dramatically in vaccinated women, but not entirely eliminated. In addition, some women will not develop a protective immune response following vaccination or may not be vaccinated for a variety of reasons.

Based on statistical analysis of HPV type distributions in populations, it appears unlikely that reducing the frequency of specific HPV types in a population through vaccination would lead to an increase in the frequency of other HPV types (M. Schiffman, unpublished observation). Therefore, it is expected that vaccination of adolescents would lead to a reduction in rates of HPV infection that would be manifested initially as lower rates of low-grade SIL as women initiate sexual activity. As this group ages, a reduction in the expected rates of high-grade SIL and then carcinoma would be expected. Ultimately, the impact of vaccination on rates of high-grade SIL and carcinoma would be expected to exceed that found in low-grade SIL because the vaccine would be directed against cancer-associated HPV types that are overrepresented in high-grade SIL and cancer. During this period, the ratio of specific cytologic diagnoses in the population would be in a state of flux.

Over time, the SILs remaining in the population would be related increasingly to HPV types that are less likely to persist and to progress to cancer. Consequently, there would be fewer SILs in the population, and the SILs remaining would be more likely to spontaneously regress without treatment. For example, the lifetime risk for developing carcinoma in women with low-grade SIL is estimated at about 1%.[24] Effective elimination of the most oncogenic HPV types in a population might reduce this risk to 1 in 500 or 1,000. At this level, it is unlikely that low-grade SIL would require treatment. Emphasis would need to be placed on the detection of high-grade SIL.

Theoretically, a vaccine program could also reduce risk among nonvaccinated women because the chance of acquiring a cancer-associated type of HPV infection with each new sexual partner would be reduced. This effect would be most dramatic in high-risk populations in which even a single sexual contact in a setting without HPV vaccination conveys a large risk for HPV infection. By reducing the risk per sexual contact, vaccination could establish a linear relationship between cancer risk and number of sexual partners, as seen in low-risk populations (e.g., middle-class women in the United States). However, realization of this prediction would require vaccination of women in all demographic groups within the population, because sexual contacts may be demographically restricted.

Cost-effective cancer prevention in an HPV-vaccinated population would probably require targeted screening of women just a few times during an entire lifetime, rather than annual screening. HPV DNA testing and perhaps serology could be useful in identifying women at risk. Ideally, the result would be more effective screening following lengthier intervals, with a reduction in cost and morbidity associated with treatment of precursors. The role of cytology in such a setting is unclear. Manual screening of conventional smears would become increasingly tedious as the number of abnormal cases declines. Furthermore, lengthening of the screening interval and a greater reliance on other testing methods would reduce workloads. Although implementation of an HPV vaccination program would take decades, it will be important for the cytology community to plan for such an eventuality with foresight.

## Cervical Cancer Prevention in Older Women

Cytologic screening in older women is difficult because the transformation zone recedes into the endocervical canal, and atrophy hinders cytologic interpretation. HPV prevalence declines sharply with age, dipping below 5% in women over 45. Consequently, overdiagnosis of reactive or atrophic changes as SIL increases with age,[25] adding to the screening problem in these women. Nonetheless, rates of invasive cervical cancer remain stable in these patients.

Prophylactic HPV vaccines are unlikely to be cost-effective in older women because acquisition of new HPV infections is uncommon. However, HPV infection in older women probably implies a greater disease risk than in young women. Most HPV infections in young women reflect recent

acquisition related to a new sexual contact and regress spontaneously. Because older women generally do not make new sexual contacts, HPV DNA detection is more likely to signify viral persistence related to an ineffectual host immune response and, consequently, a greater risk of disease. This assumption, in combination with the low prevalence of HPV infection in the elderly, suggests that HPV testing may be useful in this age group.

Older women who are cytologically normal and HPV DNA-negative may not require additional cytologic surveillance, because both cancer risk and the chance of acquiring a new HPV infection are low. Women who test positive for cancer-associated types of HPV DNA would be candidates for colposcopy. In older women, emphasis could be placed on detecting and treating prevalent disease and lengthening the screening interval in women who test negative for HPV DNA. As the first generation of young women who have been successfully vaccinated ages, it may possible to reduce testing in older women even further.

## Conclusions

It is likely that prophylactic HPV vaccine trials will be conducted in the near future with the initial intent of demonstrating safety and optimizing administration protocols. Widespread implementation of an HPV vaccine program, however, is unlikely to occur until the next century, and its impact would not be fully appreciated for decades. Meanwhile, continued efforts focusing on improving existing cervical cancer prevention programs are needed.

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